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THE JOURNAL

OF

APPLIED BACTERIOLOGY

EDITED FOR THE SOCIETY

BY

F. A. SKINNER, C. H. COLLINS

AND

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VOLUME 54, 1983

Published by Blackwell Scientific Publications Ltd,

Oxford, London, Edinburgh, Boston and Melbourne

Printed in Great Britain

Journal of Applied Bacteriology 1983, 54, 417–423

The bactericidal, fungicidal and sporicidal properties of hydrogen peroxide and peracetic acid

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Received 6 October 1982 and accepted 29 November 1982

BALDRY, M.G.C. The bactericidal, fungicidal and sporicidal properties of hydrogen peroxide and peracetic acid. *Journal of Applied Bacteriology* 54, 417–423.

The antimicrobial properties of aqueous solutions of peracetic acid and hydrogen peroxide have been compared. Peracetic acid exhibited excellent antimicrobial properties, especially under acidic conditions. Reductions by a factor of 10^6 in the numbers of vegetative bacteria are obtained within 1 min at 25°C using a solution containing 1·3 mmol/l of peracetic acid. Rapid activity against bacterial spores and yeasts also occurs. Hydrogen peroxide is more effective as a sporicide than as a bactericide, with sporicidal action being obtained using a solution containing 0·88 mol/l. Bactericidal action is poor but hydrogen peroxide was bacteriostatic at concentrations above 0·15 mmol/l.

The antimicrobial properties of peroxygen compounds have been recognised for many years and a variety of applications have been developed. Dilute solutions of hydrogen peroxide are used as antiseptics (Gump 1979) and the sporicidal activity of this substance (Curran *et al.* 1940; Swartling & Lindgren 1968; von Bockelmann & von Bockelmann 1972; Ito *et al.* 1973; Toledo *et al.* 1973; Wardle & Renninger 1975) has led to use in aseptic packaging techniques. Peracetic acid is reported to be a most effective biocide and is used, both in aqueous solution and as an aerosol or vapour, for sterilisation in gnotobiotic units (Greenspan *et al.* 1955; Doll *et al.* 1963). It has also been proposed as a sterilant for heat-sensitive equipment (Bansemir *et al.* 1979) and as an anti-viral agent (Kline & Hull 1960). It is used for the disinfection of brewery equipment and similar plant. However, the variety of experimental methods adopted prevents comparison of the activities of hydrogen peroxide and peracetic acid against organisms of relevance in the various applications described above.

0021–8847/83/0600–0417 $02.00

Experiments have therefore been carried out to enable the bacteriostatic, bactericidal and sporicidal activities of hydrogen peroxide and peracetic acid to be compared. The activity of peracetic acid against yeasts was also determined.

Materials and Methods

MICRO-ORGANISMS

All the micro-organisms used were standard strains. The bacteria were obtained from the American Type Culture Collection, Rockville, Maryland, USA. The yeasts were supplied by The National Collection of Yeast Cultures, Norwich, UK. The micro-organisms were maintained by serial sub-culture, fresh cultures being started at monthly intervals from stock cultures. Asporogenous bacteria were sub-cultured daily in Nutrient Broth (Oxoid) whilst spore-formers were treated similarly in a sporulation broth containing 10 g/l Bactopeptone (Difco) and 20 mg/l manganese (II) sulphate. Yeasts were sub-cultured twice per week on Wort Agar (Oxoid). All experiments were performed in duplicate.

CHEMICALS

The peroxygen compounds tested were commercial solutions of hydrogen peroxide (35% m/m) and peracetic acid (36% m/m), both manufactured by Interox Chemicals Ltd. The hydrogen peroxide is stabilised on manufacture, as is the peracetic acid, which also contains hydrogen peroxide (4% m/m), sulphuric acid (1% m/m), acetic acid and water.

The deactivators used were sodium thiosulphate pentahydrate (2 g/l) for peracetic acid and catalase (0·25 g/l of Sigma product C-10) for hydrogen peroxide. All chemicals used were of AnalaR grade unless suppliers' names are given.

BACTERIOSTATIC ACTION

For testing bacteriostatic activity, a 24 h culture of bacteria was diluted in sterile quarter-strength Ringer solution (Oxoid) and added, to give a final concentration of 10^4 cfu/ml, to a growth medium containing 7 g/l bactopeptone, 5 g/l glucose, the biostat under test and sufficient amounts of di-sodium hydrogen orthophosphate dodecahydrate and citric acid to buffer at the desired pH.

The systems were incubated at 37°C for 5 d and then examined visually for signs of growth, i.e. turbidity.

BACTERICIDAL AND FUNGICIDAL ACTION

To determine activity against bacteria, a solution of the biocide under test was prepared in quarter-strength Ringer solution, maintained at the desired pH by the citrate-phosphate buffer. A 24 h (10 d for spore-formers) culture of bacteria, diluted in quarter-strength Ringer solution, was added to give 10^6 cfu/ml and at various time intervals 1 ml samples were transferred aseptically to 10 ml volumes of Nutrient Broth No. 2 (Oxoid) containing an appropriate deactivator. After incubation at 37°C for 3 d (21 d for spore-formers), the media were examined for signs of growth.

The activity of peracetic acid against yeasts was evaluated similarly. Before the experiments the yeasts were given two 24 h sub-cultures in Sabouraud Liquid Medium (Oxoid), followed by a 48 h sub-culture in this medium, which was then used to form the inoculum in an experiment. Sabouraud Liquid Medium with deactivator was used as the growth/recovery medium, the cultures being incubated for 7 d at 25°C.

The effect of the repeated exposure of yeasts to sub-lethal concentrations of peracetic acid was also determined. Eight serial sub-cultures (two per week) were performed by adding 1 ml of yeast suspension to 10 ml of a solution containing 0·47 mmol/l of peracetic acid (and also, of necessity, 0·12 mmol/l of hydrogen peroxide). After exactly one minute, 1 ml of this suspension was used to inoculate 10 ml of fresh Sabouraud Liquid Medium. As deactivators were not used the Sabouraud Liquid Medium contained residual peracetic acid and hydrogen peroxide at concentrations of 47 μ mol/l and 12 μ mol/l respectively.

SPORICIDAL ACTION

Sporicidal action was also determined using bacterial spores dried on carriers. The carriers used were stainless steel rings of 15 mm length, 10 mm outside diameter and 2 mm wall thickness. These were cleaned before use by washing with Triton X-100 (BDH Chemicals), followed by thorough rinsing. They were then sterilised by heating to 180°C for 3 h in an oven. A 10 d culture of spore-forming bacteria (10 ml volumes) was shaken on a vortex mixer with glass balls of 1·5–2·0 mm diameter to assist in the disintegration of the pellicle. Four cooled, sterile carriers were placed in each 10 ml volume of bacterial culture and left to stand for 15 min. The carriers were then removed from the suspension and dried over calcium chloride *in vacuo* for 24 h. Samples (12 ml) of the system under test were transferred into sterile test tubes. The pH of the solutions was controlled, when desired, by standard buffer powders (Electronic Instruments Limited, EIL). Five dried, contaminated carriers were placed in each aliquot and left for the required time. The carriers were then transferred aseptically to individual tubes containing 10 ml of Nutrient Broth No. 2 and deactivator. The media were incubated for 21 d at 37°C prior to inspection.

Results

Peracetic acid and hydrogen peroxide were found to have comparable bacteriostatic activities (Table 1) although peracetic acid is less effective at pH 8. The generally greater sensitivity to pH of peracetic acid is relatable to the lower pk_a (8·2) compared with that of hydrogen

Table 1. Bacteriostatic activities of hydrogen peroxide and peracetic acid

Biostat	pH	M.i.c. (mmol/l) *Pseudomonas aeruginosa* ATCC 15442	*Klebsiella pneumoniae* ATCC 4352	*Streptococcus faecalis* ATCC 10541	*Staphylococcus aureus* ATCC 6538
Hydrogen peroxide	5·0	0·15	0·75	0·75	0*
	6·5	0·30	0·75	0·75	0·15
	8·0	1·50	0·75	0·75	0·15
Peracetic acid	5·0	0·33	0·33	0·33	0
	6·5	0·33	0·33	0·33	0·33
	8·0	0·66	0·66	0·66	0·33

* No growth in the absence of biocide.

peroxide (11·7). As with hypochlorous acid and many organic acid biocides the antimicrobial activity of the undissociated acid exceeds that of the anion formed upon dissociation. Although both peroxygen compounds are satisfactory bacteriostats, prolonged or repeated exposure to hydrogen peroxide might lead to the emergence of cells capable (due to increased catalase activity) of active growth in the presence of peroxide. This problem would not occur with peracetic acid, which is not destroyed by catalase.

Peracetic acid is an excellent bactericide (Table 2), the activity depending to some extent upon pH. Although concentrations of 66 μmol/l did not usually produce a total kill within 30 min, 330 μmol/l were effective within 10 min in all of the tests and 1·3 mmol/l always acted in under a minute. In contrast, hydrogen peroxide was found to be a weak bactericide, even a solution containing 0·88 mol/l never giving a total kill within 210 min. In this context a 'total kill' implies that, within a 1 ml sample, none of the 10^6 cfu originally present remained viable.

The results of experiments carried out to determine the activity of peracetic acid against yeasts are given in Table 3. The effect on a

Table 2. Bactericidal activity of peracetic acid

pH	Concentration (μmol/l) of peracid	Time required (min) for a complete kill *Pseudomonas aeruginosa* ATCC 15442	*Klebsiella pneumoniae* ATCC 4352	*Streptococcus faecalis* ATCC 10541	*Staphylococcus aureus* ATCC 6538
5·0	13	> 30	> 30	> 30	> 30
	66	> 30	> 30	10	30
	130	30	30	5	10
	330	5	5	5	5
	660	< 1	< 1	< 1	< 1
	1300	< 1	< 1	< 1	< 1
6·5	13	> 30	> 30	> 30	> 30
	66	> 30	> 30	10	> 30
	130	30	30	10	30
	330	10	5	5	5
	660	< 1	< 1	< 1	5
	1300	< 1	< 1	< 1	< 1
8·0	13	> 30	> 30	> 30	> 30
	66	> 30	> 30	> 30	> 30
	130	30	10	5	30
	330	10	10	5	10
	660	< 1	< 1	5	5
	1300	< 1	< 1	< 1	< 1

Table 3. Action of peracetic acid on yeasts

pH	Concentration (mmol/l) of peracid	Time required (min) for a complete kill								
		Saccharomyces cerevisiae NCYC 762			*Saccharomyces cerevisiae* NCYC 1026			*Zygosaccharomyces bailli* NCYC 580		
		a	b	c	a	b	c	a	b	c
5·0	0·13	> 30	> 30	> 30	> 30	> 30	> 30	> 30	< 5	> 30
	0·40	> 30	> 30	> 30	20	30	> 30	10	< 5	> 30
	0·66	10	< 5	> 30	< 5	< 5	> 30	< 5	< 5	> 30
	1·3	< 5	< 5	> 30	< 5	< 5	> 30	< 5	< 5	> 30
	6·6	< 5	< 5	10	< 5	< 5	< 5	< 5	< 5	10
	13	< 5	< 5	< 5	< 5	< 5	< 5	< 5	< 5	< 5
6·5	0·13	> 30	> 30	> 30	> 30	> 30	> 30	30	> 30	10
	0·40	> 30	> 30	> 30	> 30	> 30	> 30	< 5	30	< 5
	0·66	30	> 30	> 30	< 5	10	> 30	< 5	< 5	< 5
	1·3	< 5	< 5	> 30	< 5	< 5	20	< 5	< 5	< 5
	6·6	< 5	< 5	< 5	< 5	< 5	< 5	< 5	< 5	< 5
	13	< 5	< 5	< 5	< 5	< 5	< 5	< 5	< 5	< 5
8·0	0·13	> 30	> 30	> 30	> 30	> 30	> 30	10	20	10
	0·40	> 30	> 30	> 30	> 30	> 30	> 30	< 5	< 5	< 5
	0·66	> 30	> 30	> 30	> 30	10	> 30	< 5	< 5	< 5
	1·3	10	30	> 30	< 5	< 5	> 30	< 5	< 5	< 5
	6·6	< 5	< 5	10	< 5	< 5	< 5	< 5	< 5	< 5
	13	< 5	< 5	< 5	< 5	< 5	< 5	< 5	< 5	< 5

a, at 25 C; b, at 25 C after repeated exposure to peracetic acid; c, at 4 C.

brewing yeast, *Saccharomyces cerevisiae* NCYC 1026, and on two typical infecting 'wild' yeasts was measured. Since a variety of temperatures are encountered in breweries, experiments were carried out at both 4 C and 25 C. The yeasts were also exposed repeatedly to sub-lethal concentrations of peracetic acid and then retested to ascertain if an increased resistance to peracetic acid had developed.

The results of the experiment performed at 25 C show that the yeast strains vary in their resistance to peracetic acid, with the wild strains NCYC 762 and 580 being respectively the most and least resistant.

It is also apparent that the efficacy of peracetic acid against the two strains of *S. cerevisiae* decreases with increasing pH. The yeasts exposed repeatedly to sub-lethal peracetic acid concentrations did not exhibit any great difference in their resistances to the biocide. Whilst it is impossible to demonstrate that resistance will never develop, these results strongly suggest that any loss of sensitivity to peracetic acid will not develop easily or rapidly. The efficacy of peracetic acid is much less at lower temperatures. This is not surprising, as disinfection, like other chemical processes, almost invariably takes place at a slower rate as the temperature falls.

Table 4. Sporicidal action as measured by the suspension test

Biocide	Biocide concentration (mmol/l)	Time required (h) for complete kill of *Bacillus subtilis* ATCC 15441		
		pH 5·0	pH 6·5	pH 8·0
Peracetic acid	1·3	> 6	> 6	> 6
	13	1	3	> 6
	130	< 0·5	< 0·5	< 0·5
	380	< 0·5	< 0·5	< 0·5
Hydrogen peroxide	290	> 6	> 6	> 6
	880	3	6	6

The results for *Zygosaccharomyces bailli* were variable but this species was generally the most sensitive whilst *S. cerevisiae* NCYC 762 remained the most resistant.

Tests were performed to measure the activity against bacterial endospores by two methods. The results of a suspension test, similar to that used to determine activity against yeasts and vegetative bacteria, are shown in Table 4. Peracetic acid is an excellent sporicide, a 130 mmol/l solution being effective in 30 min within the pH range 5·0–8·0. Even a 13 mmol/l solution sterilises within 1 h at pH 5·0. Hydrogen peroxide is less effective, but still acts as a sterilant, a 0·88 mol/l solution acting within 3–6 h. A similar time was found to be required for a commercial chemosterilant based upon 2% glutaraldehyde in alkaline solution.

A more stringent test of sporicidal activity was carried out by means of a carrier test in which the spores were dried onto stainless steel carriers prior to exposure to the biocide. In this test the spores were protected from the biocide not only by the carriers themselves but also by dried residues from the sporulation broth.

Results in Table 5 show that a 0·29 mol/l solution of hydrogen peroxide is not a sterilant although 0·88 mol/l for 6 h is effective over a wide range of pH values. However, a shorter contact time, 4 h, is insufficient to cause satisfactory sporicidal activity. Peracetic acid is a very effective sporicide, a 39 mmol/l solution killing virtually all the spores within 30 min. It should be noted that growth will occur if only one spore survived to germinate and multiply within the 21-day incubation period.

Discussion

The results are generally in agreement with the data in the literature. Peracetic acid is an excellent bactericide, fungicide and sporicide. As expected, a higher dose is required to kill spores than vegetative bacteria. For example, in the suspension test at pH 5·0, a sporicidal dose is 13 mmol/l for 60 min, whereas bactericidal action requires only 0·13 mmol/l for 30 min. In contrast, hydrogen peroxide is more effective as a sporicide than as a bactericide. A solution containing 0·88 mol/l was sporicidal within 3 h at

Table 5. Sporicidal activity as measured by the carrier test

Sporicide	Concentration (mmol/l)	Contact time (h)	pH	Non-sterile carrier* ratio
Hydrogen peroxide	290	6	5·1	15/15
	290	24	5·1	10/15
	880	4	4·3	9/15
	880	6	4·3	0/15
	880	17	4·3	2/15
	880	24	4·0†	1/14
	880	24	4·3	0/24
	880	24	7·0†	1/14
	880	24	9·0†	1/14
Peracetic acid	13	24	3·1	13/30
	39	0·5	2·9	1/15
	39	1	2·9	0/15
	39	2	2·9	2/15
	39	4	2·9	1/12
	39	6	2·9	3/15
	39	24	2·9	1/12
	130	1	2·6	1/15
	130	24	2·6	1/11
	390	24	2·3	1/15
	390	24	4·0†	0/11
	390	24	7·0†	0/11
	390	24	9·0†	0/11

* The numerator is the number of carriers which were found to retain viable spores: the denominator is the number of carriers tested.

† Buffer present.

pH 5·0 and 6 h at pH 6·5, but no corresponding bactericidal action was noted at either pH using a contact time of 3·5 h. Results obtained by Wardle & Renninger (1975) reflected a similar sporicidal action (a 0·88 mol/l solution killing all spores, initially at 10^7 cfu/ml, within 150 min at an unspecified pH) but a much better bactericidal action, a 0·88 mol/l solution producing a similar reduction in the numbers of *Micrococcus* spp. and *Staphylococcus epidermidis* within 10 min. Amin & Olson (1968), however, reported that 14·7 mmol/l of hydrogen peroxide killed only 99·9% of cells of *Staph. aureus* at an initial concentration of 10^5 cfu/ml in 171 min. The bactericidal activity of hydrogen peroxide is not related to catalase activity (Ahmed & Russell 1975) and is thought to occur as a result of multiple cellular injuries which can be repaired to a certain extent (Campbell & Dimmick 1966). It is possible that in the present work the experimental conditions were such that these repair mechanisms were able to ensure the viability of at least a few cells. Findings summarised by Russell (1982) suggest that hydrogen peroxide exerts sporicidal activity by removing protein, presumably from the coat, although an initial non-lethal removal of the exosporia may also take place (Ahmed & Russell 1975).

Some evidence for the reduction by organic matter of the antimicrobial activity of peracetic acid may be seen in that, at pH 5·0 for instance, a 0·13 mmol/l solution is bactericidal but the m.i.c. is 0·33 mmol/l. An obvious explanation for this is that the m.i.c. is increased by chemical reactions between the added peracetic acid and organic nutrients in the bacteriostatic test. These nutrients are not present under the conditions of the bactericidal test.

Peracetic acid is corrosive towards some metals and is toxic. (Figures cited by Lewis & Tatken (1980) include, for example, a rat oral LD_{50} of 1540 mg/kg.) For certain applications, however, the rapid activity of peracetic acid against a variety of micro-organisms is such as to merit consideration of this chemical as a disinfectant or sterilant. Problems of corrosivity could be reduced by using commercial formulations containing a lower concentration of peracetic acid and no sulphuric acid, and which are therefore suitable for use with stainless steel equipment. These formulations contain higher proportions of hydrogen peroxide and this might allow the use of peracetic acid concentrations lower than those required when the 36% peracetic acid is used.

The author would like to thank the management of Laporte Industries Limited and Interox Chemicals Limited for permission to publish this article, and S.R. Mearns and G.L. Worrall for their technical assistance.

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Critical Reports on Applied Chemistry Volume 23

Industrial Biocides

Edited by K. R. Payne

Published on behalf of the Society of Chemical Industry by
John Wiley & Sons
Chichester · New York · Brisbane · Toronto · Singapore

Library of Congress Cataloging-in-Publication Data:
Industrial biocides.
(Critical reports on applied chemistry: v. 23)
1. Pesticides. 2. Disinfection and disinfectants.
3. Chemical preservatives. I. Payne, K. R. (Kenneth Richard) II. Society of Chemical Industry (Great Britain) III. Series.
TP248.P47I52 1988 668'.65 87-37142

ISBN 0 471 91880 6

British Library Cataloguing in Publication Data:
Industrial biocides.
1. Industrial microorganisms. Control. Use of in biocides.
I. Payne, K. R. (Kenneth Richard, *1920–*
660'.62

ISBN 0 471 91880 6

Typeset by Photo-Graphics Ltd., Honiton, Devon.
Printed and bound in Great Britain by Biddles Ltd., Guildford, Surrey.

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6 Disinfection with peroxygens

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6.1 Introduction

The increasing desire for improved hygiene and sanitary conditions has led to a greater application and use of peroxygens throughout the world. This review examines critically hydrogen peroxide, peracetic acid formulations, other peroxyacids and inorganic peroxygen compounds from the perspectives of chemical properties, biocidal properties and industrial applications. At the end of each sector case histories are highlighted to exemplify and demonstrate practical applications.

6.2 Hydrogen peroxide

6.2.1. Properties

Properties of hydrogen peroxide (35% w/w) which contribute to its suitability in environmental protection include stability for prolonged storage, ease of handling, non-corrosivity, strong oxidizing properties, total miscibility with water and convenience of dosing with readily available equipment. It can assist in biological oxidation processes [1,2] and is non-polluting, decomposing to oxygen and water.

Hydrogen peroxide is used widely for odour control in sewage and sewage sludges [3, 4] and in the purification of landfill leachates [5, 6] through selective microbial control of anaerobic sulphate-reducing bacteria, a broad spectrum of filamentous bacteria and other organisms.

Other important disinfection applications include medical uses, swimming pool cleansing, horticulture, as well as sterilization of carton materials in aseptic packaging of milk, juices, wines and a wide range of beverage products. The applications of hydrogen peroxide in industrial effluent treatment have been reviewed widely [7, 8].

6.2.2 Biocidal properties

Concentrations of hydrogen peroxide as low as 25 mg/l are able to prevent the growth of many bacteria. Hydrogen peroxide is capable of destroying bacterial spores at higher concentrations, where it is used to provide sterile conditions (Table 6.1).

It should be noted that a contact time of several hours is needed to achieve sterility using 30 g/l hydrogen peroxide. However, shorter contact times are

Table 6.1 Effect of 30 g/l hydrogen peroxide in pH 6.5 buffer on spores of *Bacillus subtilis* NCTC 10452 at ambient temperature

Time (min)	Viable spores per ml
0	1.9×10^5
60	300
120	300
180	7.4
240	0
300	0

suitable if higher concentrations of hydrogen peroxide are used or if hydrogen peroxide is used in conjunction with ultraviolet radiation or with moderate heat. Several reviews have illustrated increased sporicidal effects of hydrogen peroxide with both of these phenomena [9, 10]. Although heat itself can kill vegetative cells, the presence of hydrogen peroxide is necessary for the destruction of spores. Aseptic packaging which involves the controlled packing of a sterile product in a sterile carton in a presterilized atmosphere is a typical example. Although similar antimicrobial properties are displayed by the solid hydrogen peroxide donors such as sodium perborate, sodium carbonate peroxyhydrate and urea–hydrogen peroxide, a limitation is that their solutions also contain the relevant organic or inorganic bases.

6.2.3 *Disinfection properties*

The antimicrobial action of hydrogen peroxide may involve impingement of surface membranes through formation of free hydroxyl radicals (OH·) [11–13] and oxidation of various groups in lipids and proteins, e.g. sulphydryl groups and double bonds [14–16]. Bacterial surfaces exposed to hydrogen peroxide have also exhibited distension and rupture [17, 18]. The impact of hydrogen peroxide on filamentous bacteria has been attributed to erosion of protective polysaccharide coatings, cleavage of cell chains [1, 2] and destruction of holdfasts [19]. Trace metal levels, e.g. iron, copper and zinc, common to organically polluted waters and often found deposited on and around growth filaments [19], serve as catalysts [18]. Examples of hydrogen peroxide in microbial control, disinfection and sterilization follow.

6.2.4 *Uses of hydrogen peroxide*

(a) Medical applications The mild disinfectant and good bacteriostatic properties of hydrogen peroxide, combined with its innocuous products of decomposition, are recognized in its wide use in hospitals and medical centres for the

treatment of cuts and wounds. Hydrogen peroxide acts against potentially harmful invading microorganisms and the oxygen evolved in this process assists the mechanical removal of dirt from the wound.

Other solid peroxygen compounds, as mentioned previously, which yield hydrogen peroxide in aqueous solutions, have been the basis of formulations used as mouthwashes and as treatment for skin infections. In these cases, the fungicidal properties are important since oral and dermal infections are frequently caused by fungi.

(b) Swimming pools In many countries hydrogen peroxide is used in conjunction with Baquacil SB (ICI trade name, a polymeric biguanide compound) for the disinfection of private swimming pools. Hydrogen peroxide improves bacterial kill, controls algal contamination, improves water clarity and can increase the efficiency of sand filtration. A major advantage of hydrogen peroxide is its ability to control algal growth, particularly in outdoor swimming pools where other biocides fail if used alone. The Baquacil/hydrogen peroxide system offers pool hygiene without the unpleasant smell, taste and eye irritation associated with the most commonly used biocide, chlorine.

(c) Horticulture Within the horticultural industry increasing use is being made of capillary feed systems for nutrient supply to plants in greenhouses. A major limitation of manual cleaning methods is that they are tedious and time consuming. Although nitric acid can be used it is phytotoxic, which limits application to the end of the growing season. In the United Kingdom major growers use hydrogen peroxide at dose levels up to 700 mg/l (as 100% w/w) for effective removal of algae causing blockage. Hydrogen peroxide and its decomposition products are non-phytotoxic and therefore cleaning is carried out on a regular basis during the growing season.

(d) Microbial growth control in streams Landfill leachates are formed when the absorptive capacity of emplaced solid wastes is exceeded on landfill sites [20–22]. Typically, leachates are laden with toxic hydrogen sulphide, are malodorous and highly polluting due to organic content and can impede tipping strategy [5]. Hydrogen peroxide is used not only for leachate treatment on site but also to control unsightly and problematic microbial growths in streams receiving discharged leachates.

Leachate was discharged from a landfill site (80 000 m^3) containing compacted paper and clay wastes for several months to a stream. Local residents had complained about lowered amenity value and unsightly congestion. Average values of determinands in the leachate were total sulphide 3 mg/l, COD 1300 mg/l, and pH 7.2. Hydrogen peroxide (35% w/w) was gravity fed from a 1 m^3 capacity storage tank to give an average concentration in the stream of 150 mg/l (as 35%). Within 10 days the benthic slime mass was removed over a

distance of 600 m and required a usage of 160 kg/day of hydrogen peroxide (35% w/w). Once heavy microbial growths had been removed, a continuous hydrogen peroxide dose of 25 mg/l (35% w/w) was used to prevent recurrence.

(e) Aseptic packaging Hydrogen peroxide (35% w/w food and drug grade) has been used in aseptic packaging applications throughout the United Kingdom for many years because of its high standards of purity and stability. Its high oxidizing properties, especially in combination with heat, result in excellent sporicidal activity. Hydrogen peroxide is favoured as the sterilant for carton material because it concentrates with heating, can be readily vapourized in hot air streams and decomposes to harmless, non-contaminating products of oxygen and water, so avoiding detrimental effects on beverage and food products. The uniform quality displayed by this grade of hydrogen peroxide and its compatibility with specialized carton components means that it can be monitored and removed with the advantage that its decomposition products are acceptable.

Packaging of beverages such as milk and fruit juices in polyethylene-coated paper containers or in plastic containers is rapidly increasing world wide. These containers are usually formed, sterilized, filled with product and sealed in a single operation on an aseptic packaging machine. The performance of hydrogen peroxide was examined by a major machine manufacturer and showed it produced a 6 to 7 logarithmic reduction from an initial bacterial count of between 0.02 and 0.05/cm^2 which included 3 per cent spores.

Although other sterilant systems such as alcohols and u.v. radiation have been considered for aseptic packaging they have been rejected on grounds of inadequate performance. However, one recently developed machine does use hydrogen peroxide (1% w/w) in conjunction with u.v. radiation. The technology of aseptic packaging by machine displays two operational methods; packs are produced either *in situ* or are preformed. Preformed packs are sterilized by spraying with hydrogen peroxide solutions followed by drying with hot air if residual hydrogen peroxide concentrations in the filled pack exceed the levels permitted by legislation. Packs may be formed *in situ* either by pressing plastic sheet or mechanically forming plastic-coated materials. Sterilization is achieved by passing the unformed sheet through a heated bath (up to 80 °C) of hydrogen peroxide (35% w/w) with a contact time of 3–9 seconds. Excess hydrogen peroxide is removed by squeeze rollers, an 'air-stream knife' or by hot air (up to 120 °C). All operations are conducted beneath an atmosphere of filtered sterile air in the machines.

6.2.5 Analytical methods

A variety of analytical procedures are available, including laboratory methods, simple tests for site and field use, and automated monitoring systems for controlled dose. Full details are available from the manufacturers.

6.2.6 *Handling/application*

Hydrogen peroxide is manufactured at concentrations up to 86% w/w which may be handled safely with suitable precautions. For most environmental applications 35% w/w concentration hydrogen peroxide is normally used. Hand and eye protection are necessary when handling the product and dilution with water is recommended for spillage. Storage may be either in or out of doors, but segregation from organic or flammable products is necessary.

The cheapest satisfactory materials for use with 35% w/w hydrogen peroxide are polyethylene or PVC. Pipework may be flexible PVC or uPVC to BS 3506 Class 7. Stainless steel (AISI grade 316) and 99.5 per cent aluminium are preferred although stainless steel should be prepassivated with nitric acid. Standard metering pumps of stainless steel, PVC or PTFE construction are satisfactory. Materials not suitable with 35% w/w hydrogen peroxide include rubber, mild steel, brass, bronze and copper. Reaction vessels for effluent treatment, where peroxide concentrations are low (e.g. less than 1000 mg/l) and usage is rapid, may be made of the recommended materials above or of concrete or mild steel.

6.3 Peracetic acid (PAA)

Throughout the food, brewing and dairy industries, use of PAA is increasing for sterilization of precleaned stainless steel storage, transfer and process vessels and soak tanks. Throughout the industrial water treatment sector demand for microbial growth control is expanding in process waters, cooling waters, recirculating water circuits and ion exchange columns. In the municipal sector peracids are being used in the disinfection of sewage effluents and sludges [23, 24].

A prime advantage of the peracids, which contain the —COOOH group is that they are non-foaming water-soluble liquids, which are fast acting, non-derivatizing, and break down to environmentally acceptable, innocuous decomposition products.

They are used over a wide temperature range against a broad spectrum of microorganisms. Depending on the end use, each formulation contains different amounts of peracetic acid, water, hydrogen peroxide, acetic acid and stabilizer to give an equilibrium mixture. Two such examples of peroxygen disinfectants are described. Application concentrations are quoted in terms of PAA content only, e.g. mg PAA/l, unless stated otherwise.

6.3.1 *Chemical properties*

Table 6.2 shows that PAA combines the active oxygen characteristics of a peroxide within an acetic acid molecule. The peracid species is believed to be primarily responsible for disinfection. The ultimate decomposition products are

Table 6.2 Typical analyses of Proxitane 1507 and Proxitane 4002, properties, status of equilibrium mixture and decomposition products of peracetic acid[a]

Proxitane 1507 content	% w/w	Properties
Peracetic acid (PAA)	15.0	Almost colourless liquid
Hydrogen peroxide	14.0	Specific gravity (20 °C) 1.12
Acetic acid	28.0	Soluble in water-polar organic solvents
Water	42.0	Freezing point −25 °C approx.
Stabilizer	<1%	Boiling point 103 °C
		Total available oxygen 9.9% w/w

Proxitane 4002 content	% w/w	Properties
Peracetic acid	38.0	Almost colourless liquid
Hydrogen peroxide	4.0	Specific gravity (20 °C) 1.135
Acetic acid	44.7	Soluble in water-polar organic solvents
Water	12.0	Freezing point −30 °C approx.
Sulphuric acid	1.0	Boiling point 105 °C
Stabilizer	<1%	Total available oxygen 9.5% w/w

Status of equilibrium mixture

$$\underset{\text{Acetic acid}}{CH_3COOH} + \underset{\text{Hydrogen peroxide}}{H_2O_2} \rightleftharpoons \underset{\text{Peracetic acid}}{CH_3COOOH} + \underset{\text{water}}{H_2O}$$

Routes of decomposition

$$CH_3C(=O)OOH + H_2O \rightleftharpoons CH_3C(=O)OH + H_2O_2 \quad (1)$$

$$CH_2C(=O)OOH \longrightarrow CH_3C(=O)OH + \tfrac{1}{2}O_2 \quad (2)$$

$$H_2O_2 \longrightarrow H_2O + \tfrac{1}{2}O_2 \quad (3)$$

[a] Proxitane is a Trademark of Interox Chemicals Limited.

hydrogen peroxide, oxygen and acetic acid, all of which are toxicologically safe at the recommended in-use concentrations and are readily soluble in water.

As PAA is fully miscible in water it is easily rinsed off, leaving a biologically clean surface. The activity of PAA, unlike that of many other biocides or sterilants, is retained in the presence of hard water and is little reduced by organic contamination such as blood, casein, faeces, serum or yeast extract. The greater ease and safety in handling and lower corrosivity of the less concentrated grades of PAA makes them particularly suitable for disinfection applications in industrial disinfection.

6.3.2 *Biocidal properties*

Peracetic acids are more potent antimicrobial agents than hydrogen peroxide [25], and are effective against a broad spectrum of microorganisms including viruses [26]. Extensive assessment has defined the performance of Proxitane 1507 against a variety of bacteria and fungi, confirming the microbiocidal efficacy of PAA.

Exposure for 10 minutes to a solution containing 0.17 per cent Proxitane 1507 (that is 250 mg PAA/l) in demineralized water resulted in the death of 10^6/ml of both gram-positive (*Staphylococcus aureus* and *Streptococcus faecalis*) and gram-negative (*Escherichia coli, Klebsiella pneumoniae* and *Pseudomonas aeruginosa*) bacteria, as well as yeasts such as *Candida albicans* and *Saccharomyces cerevisiae*. Obligate anaerobes were also killed, the oxygen produced by breakdown of the peracid being an additional factor in this case. It is also very effective against bacterial endospores (Table 6.3).

At pH 5, a 750 mg PAA/l solution killed all the spores within one hour, the shortest time period tested. Sporicidal activity was still good at neutrality. Such excellent sporicidal activity has resulted in PAA being chosen for various demanding applications, e.g. the sterilization of gnotobiotic research facilities [27, 28].

Table 6.4 shows that concentration of 50 mg PAA/l achieved 5 log-reductions in under 5 minutes on identified culture strains of yeasts. The development of resistance by yeast strains to Proxitane 1507 in either the laboratory or under operational conditions has never been observed.

In laboratory tests of the effects on *Legionella pneumophila* organisms, Proxitane 1507 was diluted in sterile distilled water in 50 ml aliquots and used immediately. Test concentrations challenged contained 6, 12, 24, 48 and 96 mg PAA/l. For the test innoculum *Legionella pneumophila* serotype 1 NCTC 11192 (Philadelphia strain) was grown in broth with constant agitation at 37 °C for 72 hours. Dilutions of the broth culture were prepared in sterile distilled water.

Table 6.3 Effect of peracetic acid on spores of *Bacillus subtilis* NCTC 10452

Time (min)	Number of residual viable spores per ml			
	375 mg PAA/l		750 mg PAA/l	
	pH 5.0	pH 7.0	pH 5.0	pH 7.0
0	1.9×10^5	2.4×10^5	1.9×10^5	2.4×10^5
60	2	300	0	22
120	1	300	0	5.5
180	0	300	0	0
240	0	288	0	1
300	0	123	0	0

Table 6.4 Action of peractic acid on yeasts at 25 °C and at pH 5.0

Concentration of PAA (mg/l)	Time required for a 99.999% kill (min)		
	Saccharomyces cerevisiae NCYC 762	*Saccharomyces cerevisiae* NCYC 1026	*Zygosaccharomyces bailii* NCYC 580
10	>30	>30	>30
30	>30	10–20	5–10
50	5–10	<5	<5
100	<5	<5	<5
500	<5	<5	<5
1000	<5	<5	<5

Suspensions of 1 ml were added to each 50 ml aliquot of diluted biocide and to a 50 ml aliquot of distilled water (control). Test mixtures, which were agitated intermittently, were incubated at 23 °C in dark conditions. Samples (2 × 0.5 ml) of each test were taken at 1-hour, 6-hour and 24-hour intervals and neutralized.

The number of viable *L. pneumophila* were reduced from 4×10^6 cfu/ml to zero at all test concentrations ranging from 6 to 96 mg PAA/l. Even after 17 days incubation all plates remained sterile (Table 6.5).

6.3.3 *Disinfection*

Proxitane 1507 and 4002 are extremely fast acting over a wide range of temperatures (−30 to +70 °C) and so remain highly effective whether used in enclosed clean-in-place (c.i.p.) systems or outdoor applications where greater variations in ambient temperature are encountered.

Investigations have shown that PAA has a much stronger biocidal action than either hydrogen peroxide or acetic acid from which it is derived and to

Table 6.5 Action of peracetic acid on *Legionella pneumophila* serotype 1

Sample	Concentration (mg PAA/l)	Incubation time (hours)	cfu/ml[a]
Control	—	1	4.0×10^6
Control	—	24	3.8×10^6
	6	1	Sterile
	12	1	Sterile
	24	1	Sterile
	48	1	Sterile
	96	1	Sterile

[a] Colony-forming units/ml.

which it ultimately decomposes [25, 29]. Also, PAA has demonstrated the highest kill rate at the lowest concentration when compared with a broad battery of other available disinfectants tested in the vapour phase [30].

PAA probably disrupts sulphhydryl (—SH) and sulphur (S—S) bonds within enzymes; hence important components in membranes are broken by oxidative disruption [31]. It is likely PAA dislocates the chemiosmotic function of membrane transport through rupture or dislocation of cell walls, which seriously impedes cellular activity. Intracellular PAA may also oxidize essential enzymes; thus vital biochemical pathways, active transport across membranes and intracellular solute levels are impaired [23]. An important point is that PAA is unaffected by and inactivates catalase, an enzyme known to detoxify hydrogen peroxide [32]. One reason why anaerobes, e.g. sulphate-reducing bacteria, are so sensitive to PAA is that they lack both catalse and superoxide dismutase. The ovicidal properties of PAA may be related to its effects as a protein denaturant, demonstrated when used as a sporicide [23]. Lack of visible movement, dark coloration, granulation and ovoid shrunken appearance are typical symptoms among cestode oncospheres in sewage sludges disinfected with PAA. One explanation of this mechanism is that PAA alters the chemical constitution and so weakens the cement bonding between embryophoric blocks, before penetrating more deeply. It was observed that a dose of 250 mg PAA/l in a digested sludge was as ovicidal as 1000 mg PAA/l in a raw sludge, and killed up to 99 per cent of the embryos, as diagnosed from current methods of viability determination.

6.3.4 Brewing industry

Important trends in the brewing industry include more continuous brewing processes, increase in plant size, liquor conservation and automated cleaning programmes for greater efficiency, control and safety. For many years caustic cleaning cycles followed by a chlorine-based disinfectant have been widely used for sanitization of batch brewing equipment. Such cleaning can form scale which reduces heat transfer through exchangers. These problems often arise from the need to maintain a carbon dioxide atmosphere in continuous brewing. Hence, a significant trend is towards acid cleaning regimes which avoid scale decomposition, lower detergent use and strip salts from equipment walls.

Proxitane 1507 is used at ambient temperatures, often in the presence of CO_2. Terminal disinfection with PAA has demonstrated benefits of decreasing down-time, reducing load to effluent plant and stripping of organic stains from inside pipe walls and vessels. PAA has proved highly effective against a wide range of microorganisms problematic to brewing processes under operational conditions [25, 33, 34]. Organisms eradicated or controlled include both bacterial vegetative cells and spores and various yeasts [35–38]. Plant does not require a final liquor rinse if borehole supplies of such liquors are known to be contaminated. Any traces of acetic acid remaining are insignificant in

Table 6.6 Recommended application concentrations for clean-in-place systems

Temperature (°C)	Contact time (mins)	Concentration of Proxitane 1507 (%)	Concentration of PAA (mg/l)
<8	15–20	0.1–0.7	15–1050
8–20	10–15	0.05–0.2	75–300
20–30	5–10	0.05–0.2	75–300
30–40	5	0.05–0.15	75–225
40–70	5	0.05–0.1	75–150

comparison to the concentrations naturally present in beers, so taste is not affected and taint does not occur.

Recommended application concentrations for clean-in-place (c.i.p.) systems are noted in Table 6.6.

(a) Automated sterilization At a major brewery, an automated system using Proxitane 1507 was requested in the fermentation vessel (FV) department, which contained 56 cylindricoconical (14 500 litre capacity) vessels.

The sterilant was metered automatically into the reclaim tank (10 000 litre capacity). Diluted Proxitane 1507 (280–300 mg PAA/l) was circulated by means of the in-place-clean (i.p.c.) pump through the designated number of FVs prior to scavenging and reclaim in the dilute sterilant tank. This operation occurred 10–20 times per day, and was monitored continuously by the Interox 242 analyser.

To avoid any time delay between dilute sterilant leaving the storage tank and a measurement being obtained, a fast simple loop was installed from the tank to a point close to the analyser and back. In a side-line to the analyser a Hallikainen self-cleaning filter was installed to avoid yeast deposits interfering with analyser operation. This sampling system connected to the analyser allowed the concentration of the stored dilute PAA to be monitored continuously.

A control unit was designed and installed to use the continuous signal from the monitor to activate a feed pump which delivered Proxitane 1507 from the storage tank to the dilute sterilant tank. The control unit was operated by main timers to ensure that the feed pump continuous activation was controlled to a maximum of 2 h. During cleaning operations, main timers made sure the feed pump would not be inactive for more than 10 h. The control unit, activated by 4–20 mA output from the monitor, controlled a Nikisso model BZ–5 pump operating at 5 l/h. This feed pump added the required amount of concentrated sterilant to the reclaim tank to keep the sterilant solution in the concentration range required.

(b) Disinfection of FVs A small private brewery encountered persistent problems of infected wort prior to fermentation in its stainless steel non-pressure

Table 6.7 Clean-in-place regime of fermentation vessels in a small brewery

1.	Hot water rinse at 85 °C
2.	Caustic detergent (2% v/v) for two recycles each of two minutes duration followed by discharge to drain, to remove particulate soiling material
3.	Caustic detergent (2% v/v) at 85 °C for three recycles each of two minutes duration followed by reclaim
4.	Hot water rinse at 85 °C to remove caustic residues
5.	Sterilization using steam

vessels, each of which was 45 000 litres capacity. Details of the initial c.i.p. regime are shown (Table 6.7).

Proxitane 1507 at a concentration of 0.2% v/v (300 mg PAA/l) was in-line injected into the c.i.p. unit during the final rinse stage at 85 °C. A contact time of 2 minutes achieved excellent bactericidal results. The renewal of hatch seals prevented odour, while a reduction in temperature to 45 °C with the same concentration and contact time achieved excellent bactericidal results, and avoided the need for subsequent cold water rinsing.

6.3.5 *Dairy industry*

Throughout the Western World great emphasis is being focused on a more cost-effective use of resources in the dairy industry because of the elevated costs of manpower, energy and need to improve sanitization standards. For example, in the United Kingdom, this has led to the opening of some very large factory complexes with a multipurpose function in processing a wide diversity of products. The need for cold terminal sterilization with Proxitane 1507 is expanding because of the increased use of stainless steel storage silos and process tanks, together with fully automated c.i.p. systems throughout the reception, storage and process arenas. The many advantages offered by Proxitane 1507 include a lower rate of application and consumption in comparison to proprietary hypochlorite brands, considerably reduced corrosivity, avoidance of terminal liquor rinse, use at ambient temperature and so decreased energy costs for steam sterilization.

Important variables affecting operational disinfection of road tankers with Proxitane 1507 do not differ from those of chlorine-based sterilants. It is important that precleaning liquors are well circulated and make contact with the interiors of drain valves, pipes and vessel surfaces. Mechanical variables of importance to the success of disinfection include: adequate water pressure, non-leakage of sterilant in circulation, sound pipe connections and functional sprayballs. Operators must recognize mechanical problems when they occur and rectify them accordingly. Some case histories follow.

(a) Disinfection of road tankers At a large processing dairy with an intake of 900 000 litres of milk per day, Proxitane 1507 was evaluated as the terminal

Table 6.8 Clean-in-place regime and terminal sterilization of road tankers

Clean-in-place regime on Cipal T	Terminal sterilization regime*	
1. Raw milk discharged to balance tank	1. Pump on	30 s
2. Cold water rinse	2. Scavening	105 s
3. Caustic detergent (70 °C)	3. Pump on	75 s
4. Cold water rinse	4. Recycle	120 s
5. Terminal sterilization regime*	5. Scavenging	85 s

disinfectant on 3 Alfa Laval Cipal T units in the road tanker bay where raw milk is received. Details of the c.i.p. regime and the terminal disinfection regime are shown (Table 6.8).

Proxitane 1507 was injected into the final rinse cycle using Nikisso BZ–30 stainless steel pumps at doses of 120–200 mg PAA/l. Results were compared to those achieved by free available chlorine (FAC) values of 200 mg/l applied identically using sodium hypochlorite (12% NaOCl). Routine sampling, over several weeks when applied levels were lowered, revealed that concentrations of 100 mg PAA/l gave consistently lower total viable counts (t.v.c.s) than FAC values of 150 mg/l at all ambient temperatures.

Consistently improved microbiocidal performance of Proxitane 1507 at lower doses than sodium hypochlorite, together with reduced risks of corrosion, has resulted in continuous use. Indeed, Proxitane 1507 is now used on all major processing units throughout the dairy.

(b) Disinfection and c.i.p. At a small regional processing dairy, which is currently under expansion due to depot closure elsewhere, satellite c.i.p. systems had been installed on each processing unit (Table 6.9). A potential application problem at this dairy was the diversity of flowrates with a calculated but not measured average of 9000 l/min. In-line injection of Proxitane 1507 to reach a recycle concentration of 300 mg PAA/l in the latter stages of final rinse water was used on sectors requiring disinfection more than once daily. A lower initial concentration (150 mg PAA/1) with overnight retention was used in silos.

Table 6.9 Processing units at a small dairy

Clean-in-place system	Unit	Tank No.	Capacity of each (litres)
A	Finished tanks and lines	5	13600
B	Silos	5	68000
	+ raw milk lines + coolers + tank	1	27000
C	All other raw milk containers	4	13600

6.3.6 *Industrial water circuits*

Ideal conditions for the growth of a wide range of microorganisms occur frequently in industrial water circuits. Wind distributed microorganisms continuously contaminate open evaporative systems which cool large volumes of water. Additional nutrients can also be made available by the use of scale and corrosion control chemicals. These can provide sources of phosphate and products that degrade to produce phosphates.

The occurrence of many microorganisms can lead to a wide spectrum of problems if left uncontrolled. Growths of microorganisms on internal surfaces can lead rapidly to the restriction of water flow in narrow waterways, e.g. compressors. Inorganic materials from the water are incorporated, often into the microorganism matrix, giving rise to a generalized deposit that is difficult to remove. Ideal conditions for the accumulation of complex floating mats of algal growth may also occur in areas of stagnant water, e.g. along the edges of cooling tower basins. Such growths may cause major blockages in pump inlets. Reductions in heat-transfer coefficient of 20–90 per cent can occur on heat-transfer surfaces with generalized growth of microorganisms, especially when inorganic debris is incorporated into the organic matrix. Such growths result in efficiency loss and increased operating costs. When biofouling deposits reduce oxygen diffusion to metal surfaces, galvanic corrosion cells are formed due to differential oxygen concentrations and corrosion ensues rapidly. If anaerobic conditions at the metal surface occur from heavy microbial deposition, colonization by sulphate-reducing bacteria such as *Desulphovibrio desulphuricans* follows with the emission of toxic hydrogen sulphide and rapid localized corrosion.

A more serious problem which has immediate effects on human health is the proliferation in industrial water systems of *Legionella* serotypes. Recently, contaminated cooling systems have been implicated in many recent outbreaks of legionnaires' disease throughout the world [39]. The family Legionellaceae is ubiquitous in distribution, occurring in most natural freshwaters, e.g. lakes, rivers, streams, as well as in the soil. They proliferate especially in some man-made systems where air is used to cool water or water is used to humidify air, and are spread on water droplets. The *Legionella* group consists of resilient thermophilic organisms which are very hardy for non-spore-forming bacteria. They require iron and the protein cysteine as a nutrient source and thus coexist in close association with other slime-forming bacteria and algae which may also grant physical protection [40]. *Legionella* organisms can also survive in protozoa, e.g. amoebae, which themselves form cysts, and, consequently, can survive periods of desiccation [41]. Amongst the man-made niches in which *Legionella* thrive are calorifiers, other hot water systems below 40 °C, shower fittings and taps, cooling towers of all types and air conditioning systems [42, 43]. Systems which are maintained infrequently and rarely cleaned are prime sources of general bacterial contamination amongst which *Legionella* organisms may be present. The pathogenicity of the various serotypes, epidemiology and predis-

posing factors for infection have been well reviewed elsewhere [39]. In the United Kingdom it is estimated there are 1000 cases of Legionellosis each year, resulting in 100 deaths. Currently, intense interest is being focused on *Legionella* because of its novelty, complex epidemiology and pathogenic potential which has generated considerable alarm.

The performance and ecotoxicological properties of many biocides, currently in use for the control of bacterial growths in industrial water circuits, are limiting. Inadequate attention has been focused on the design of suitable biocide dosing regimes for effective microbial control. As *Legionella* organisms tend to thrive abundantly in spring and summer, biocidal control regimes should be seasonal to maximize cost effectiveness.

A plethora of case histories has demonstrated that Proxitane 1507 is probably the most suitable biocide currently in use. The non-selectivity and wide spectrum kill of this biocide has eradicated *Legionella* organisms associated with slimes (pseudomonads), filamentous bacterial communities, moulds and algae. At in-use concentrations it has removed slime layers, oxidized residual sludge, controlled algae and destroyed *Legionella* bacteria, whilst being neither bioaccumulatory nor giving rise to natural resistance, since Proxitane decomposition products do not add to the pollution load nor give rise to harmful intermediaries. Simple analytical test kits are readily available for on-site use by non-chemists. In the United Kingdom Proxitane 1507 has proved acceptable because its toxicity to rainbow trout (*Salmo gairdneri* Richardson) has been well defined whilst its decomposition products are environmentally acceptable. A number of case histories follow.

(a) Control of Legionella pneumophila At a major chemical manufacturing site on-site trials were conducted on five cooling towers where *L. pneumophila* serotype 6 had been identified as present throughout the system. The tower basin of each system was closed to give a concentration of 10 mg PAA/l on total system volume. Samples for bacteriological appraisal were taken immediately from each basin before and following dosing of the biocide (Table 6.10).

Table 6.10 Presence or absence of *Legionella pneumophila*

	Time				
Tower	0	20 min	40 min	1 hour	2 hours
1	Present	Absent	—	Absent	Absent
2	Present	Absent	—	Absent	Absent
3	Present		Absent	Absent	Absent
4	Present		Absent	Absent	Absent
5	Present		Absent	Absent	Absent

Legionella pneumophila was eradicated throughout each system within 20 minutes. There was an increase in surface scum attributed to elevated levels of fatty acids from the breakdown of algal cells and loosened slime deposits. Slime formers, e.g. pseudomonads, and sulphate-reducing bacteria were eliminated from all areas that had been in contact with the biocide.

(b) Another control of L. pneumophila A consumer product manufacturer was operating two open evaporative cooling towers and four air conditioning systems. Samples indicated the presence of *L. pneumophila* serogroups 3 and 6. Weekly dosing to give an *in situ* concentration of 20 mg PAA/l successfully controlled growth of these bacteria with total viable count (t.v.c.) in the system maintained consistently at <1000 organisms/ml cleaner than before. Routine testing for *Legionella* serotypes, carried out now at six-monthly intervals, have since proved negative. The system operates at pH 7.5 and total alkalinity of 100 mg/l as $CaCO_3$.

(c) Control of algae A large cooling system was operated on an amine-based scale and corrosion control regime. General bacterial growth was controlled by the amine (i.e. T.V.C.s remained in the range 10^3–10^4 organisms/ml), but slow buildup of algae occurred on the water distribution system in the tower. Water quality is shown in Table 6.11.

The application twice a year of Proxitane 1507 (20 mg PAA/l) on the total system volume resulted in rapid kill and dispersion of the algal community. A similar trial on a cooling system, which exhibited copious algal accumulation, demonstrated that a higher concentration (30 mg PAA/l) was required as a slug dose. Thereafter, lower concentrations (10 mg PAA/l) effectively controlled algal growths.

6.3.7 *Sewage sludges*

About 35 million wet tonnes of raw sewage sludge are disposed to grazing land each year in the United Kingdom [44]. Techniques of application depend on

Table 6.11 Water quality

	Units	Raw water	Recirculating water[a]
pH		7.2	7.6
Conductivity	μmhos/cm²	620	2600
Total hardness	mg/l as $CaCO_3$	332	1400
Total alkalinity	mg/l as $CaCO_3$	230	60
Chloride	mg/l as Cl^-	47	195
Concentrating factor			4.2

[a] Acid dosing employed.

land-use patterns and availability, sludge nature and transportation costs. The agricultural use of sewage sludge is likely to increase in importance in an era of dwindling resources and increasing energy costs because it provides valuable nutrients for crop or grass culture at no cost to farmers [45]. Indeed, future legislation from the European community may enforce disinfection of raw sludge as a means of stabilization prior to disposal to land.

Intestinal parasites and pathogens are often a problem associated with this route of raw sludge disposal, because conventional sewage and sludge treatment processes are not designed to control or kill pathogenic organisms [46–48]. Pathogens of especial importance include ova of the beef tapeworm (*Taenia saginata*) and salmonellae bacteria, amongst which there are a diversity of serotypes associated with raw sludges and sewage effluents [49–51].

Interox Chemicals Limited and the UK North West Water Authority (NWWA) have completed successfully a joint research programme to evaluate under field conditions suitable sludge disinfectants, one of which was Proxitane 4002. Operational use of Proxitane 4002 has reduced *Salmonella* levels by 99.999 per cent and achieved up to 99 per cent inhibition of hatching with 100 per cent destruction in viability of beef tapeworm embryos in raw sludges. Treatment costs for operational use of Proxitane 4002 can be a cost-effective means of disinfecting sludges which eradicate potential health hazards associated with both bacterial pathogens and industrial parasites. Hence, the risks of infection to farm animals can be eliminated from sludge as a vehicle of transmission, while the no-grazing interval can be reduced to only a few days, offering more efficient use of grazing land.

Trials showed Proxitane 4002 eliminated sludge septicity, reduced sludge odour and increased acceptability on mechanical spreading to pasture land, well within the application concentrations required for disinfection. Even at optimum concentrations required for disinfection of sludges, there was no harm to pasture land, beneficial organisms occurring in soil or invertebrates, while the fertilizer and soil conditioning values of sludges were undiminished. The sludge gave rise to safe, readily biodegradable, non-toxic decomposition products.

A major advantage to the water industry is that the simplicity of dosing installations avoids the need for multistage sludge treatment systems. The ease of dosing maintains operational flexibility, minimizes capital outlay and meets emergency situations. Benefits of use include compliance with fast sludge transport regimes, elimination of prolonged sludge storage, protection of food crops or surface and ground waters receiving runoff from application sites and reduction in loss of beef carcasses due to downgrading or condemnation. A case history demonstrates use.

(a) Disinfection of sewage sludge A sewage treatment works received trade effluent from agriculturally based industry including abattoir, milk processing and paper processing. Sewage was retained for *ca.* 3 hours within covered

oxygen-activated sludge tanks. Surplus activated sludge was pumped into a storage well and transferred to consolidation tanks 1 or 2 (each 276 m^3) for thickening (*ca.* 24 h retention), to achieve a solids content of 1–2% w/w. Sludge was pumped to storage tank 3 (*ca.* 48 h retention) for further thickening (3–6% w/w solids) before transfer to road tankers for disposal to pasture. Hence this works had limited storage without the capability for further sludge treatment.

Salmonella levels varied between 30 and >24 000 organisms/100 cm^3 in the consolidated sludge; hence the North-West Water Authority's Code of Practice of <1000 organisms/100 cm^3 was achieved only for 40 per cent of the operational period between February and August 1983. The random distribution of *Salmonella* levels, coupled with the extremely short retention times, meant that sludge could not be applied to land.

Once tanks 1 and 2 had been emptied and cleaned of sludge to avoid possible recontamination from residuals, Proxitane 4002 was in-line injected (250 mg PAA/l) into the main transferring surplus activated sludge. Salmonellae levels were monitored daily, before, during and after sludge thickening, using standardized techniques. *Salmonella* levels were reduced from 4622 organisms to <30/100 cm^3 of surplus activated sludge throughout all phases of sludge in both consolidation tanks following in-line injection of 250 mg PAA/l. Preliminary indicator tests had shown that at all doses between 250 and 1000 mg PAA/l, *Salmonella* levels could be reduced from 4622 organisms/100 cm^3 to <30/100 cm^3 of sludge (Table 6.12).

Table 6.12 shows these reductions were maintained throughout the entire thickening/storage system, despite an enforced retention of 5 days. It was observed that while decanted liquors had achieved remarkable clarities, treated consolidated sludge was lighter in colour (grey-brown). Septicity of settled sludge was prevented. Septicity had occurred both before and after the trial when high temperatures (24–29 °C) were recorded in the tanks.

Table 6.12 Reduction in *Salmonella* levels in tanks following in-line injection of 250 mg PAA/l

Date	Surplus activated sludge	Organisms/100 cm^3		
		Tank 1	Tank 2	Tank 3
At start	4622			
Day 1	4622	<30	<30	
Day 2	427	<30	<30	
Day 3	—	<30	<30	
Day 4	—	<30	<30	<30
Day 5	385	<30	<30	36
Day 6*	933	36	92	<30
Day 7	427	<30	<30	92
Day 6*	Ex-road tanker			<30

(b) Disinfection of sludge in tankers Proxitane 4002 was in-line injected into consolidated sludge (3.8% w/w solids) as it was loaded into road tankers (14–15 tonne loads). On each consecutive day a dose level ranging from 150 to 500 mg PAA/l was applied, and samples were taken before and after dosing of each tanker.

Table 6.13 shows that *Salmonella* levels were below the limits of enumeration in all samples taken from sludges dosed with 300 and 500 mg PAA/l. At doses below 250 mg PAA/l low levels of *Salmonella* were detactable. Even at 150 mg PAA/l *Salmonella* had been reduced from 1494 organisms/100 cm^3 to the upper range value of 427 organisms/100 cm^3 of sludge. Thus at this low dose sludge was still rendered safe for distribution to pasture land.

Table 6.13 The reduction in *Salmonella* levels following in-line dosing of PAA during loading of road tankers with consolidated sludge

Tanker loads each (12–14 tonnes)	*Salmonella* level before dosing (organisms/100cm^3)		Dose in sludge (mg PAA/l)	*Salmonella* level after dosing organisms/100cm^3)	
	Mean	Range		Mean	Range
14	560	(147 to 993)	500	<30[a]	—
9	188	(36 to 231)	300	<30	—
7	793	(143 to 2398)	250	<30	(18 to 92)
9	496	(36 to 1494)	200	50	(30 to 147)
7	730	(147 to 1494)	150	160	(30 to 42)

[a] Lowest count taken.

6.3.8 *Handling/application*

A great advantage of using Proxitane is the simplicity, ready availability and low cost of dosing equipment.

The only materials of construction for prolonged contact should be certain types of stainless steel (prepassivated with nitric acid), polytetrafluoroethylene (PTFE) or glass. Soft PVC and polyethylene are suitable for a limited time. Dosing pumps should be of the positive (piston or diaphragm) types but not peristaltic to avoid wastage through pump tube failure. An electrically activated (solenoid) valve situated close to the injection head can be used in unison with the metering pump to ensure that liquors are not forced back to the sterilant storage vessel. The solenoid valve can also prevent syphoning of PAA from the storage vessel. Alternatively, a spring-loaded antisyphon control valve, to prevent syphoning from a head, can be used as appropriate on the discharge side of the metering pump.

Confinement within discharged lines or within pumps should be avoided. A pressure relief valve and circulation loop back to the intake side of the pump

is often used to prevent pressure increase due to PAA decomposition when the dosing pump is idle for extended periods.

Before dosing pumps are switched off for any period of time they should be drained of sterilant and flushed out with clean water. If in-line injection is preferred, discharge pipes from the dosing pump should be of stainless steel with similar couplings to maximize safety on site. When dosing is intermittent a water rinse is favoured, e.g. after the last dose each day.

6.3.9 Analytical techniques

Full laboratory procedures are readily available. Of these one is suitable for the determination of peroxygen content as hydrogen peroxide and peracetic acid in dilute aqueous solutions. The principle relies on sample acidification with sulphuric acid solution when ferrous iron is added. The peroxides present oxidize the iron to the ferric state. Addition of thiocyanate produces red ferric thiocyanate complex and the peroxide is determined by spectophotometric measurement at 460 nm. A titrimetric method is suitable for the determination of product strength: hydrogen peroxide and peracetic acid solutions in the range of up to 150 g/kg (H_2O_2) and up to 400 g/kg (PAA). The principle relies on determination of hydrogen peroxide by titration with ceric sulphate solution in ice-cold sulphuric acid solution, using Ferroin as indicator. PAA is determined by the liberation of iodine following the addition of potassium iodide solution. The iodine is titrated with sodium thiosulphate solution.

Useful determinations of PAA in many field applications can now be made using a test kit (British Patent Application 85/24,625). The principle of this method is that PAA reacts with chloride ion in solution to produce free chlorine. This is determined by the formation of a red colour with *N,N*-diethyl-*p*-phenylene diamine. The colour is measured by comparison with a standard colour disc.

A further major advance has been the design and use of a peroxide analyser and control panel for controlled continuous dosing and monitoring of Proxitane 1507. The operational advantages secured by this system [52] are: reduced effort in determinations of PAA concentration in circulated solutions, reliable automated control of PAA concentration and more cost-effective use of sterilant.

6.4 Other peracids

6.4.1 Other aliphatic peracids

Although considerations of peroxyacid biocides are dominated by peracetic acid, many other organic peracids have been investigated. In principle, the peracid corresponding to any carboxylic acid can be produced, although some compounds, such as the α-hydroxy peroxyacids, are extremely unstable and cannot be isolated. Performic (HCO_3H) and perpropionic acids

($CH_3CH_2CO_3H$) have been found to display similar antibacterial activity to peracetic acid [53], but commerical development has been prevented by the greater cost of perpropionic acid compared to peracetic acid and the aggressive nature of performic acid. The use of these and similar short-chain aliphatic peracids for soil sterilization has been described [54]. More recently, longer-chain aliphatic peracids such as peroxyheptanoic and peroxynonanoic acids have been shown to have greater activity on an equimolar basis than peracetic acid, and to have far less odour, whilst still having a sufficiently high solubility in water for practical purposes. By comparison, longer-chain peracids such as perlauric acid have limited aqueous solubility [55]. These longer-chain peracids display a spectrum of antimicrobial activity that lies between that of other peracids such as peracetic acid and those of quaternary ammonium compounds, suggesting that both the hydrophobic part of the molecule and the percarboxylic acid group contribute to the observed biocidal activity.
hepatitis B and HIV (AIDS) viruses.

6.4.2 *Dicarboxylic acids*

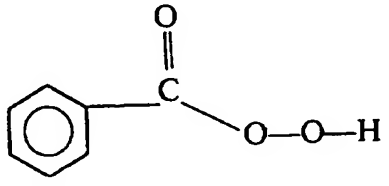
Dicarboxylic acids have also been studied. Some, such as 1–12 diperoxydodecandioic acid are stable solids, but others, such as diperoxymaleic acid, are unstable in aqueous solution. However, both mono- and diperglutaric [56] acids have been advocated as biocides, stable solutions being formed in the presence of excess hydrogen peroxide. Monoperoxyglutaric acid can also be produced from the corresponding acid peroxide in a manner analogous to the formation of monoperoxysuccinic acid from succinyl peroxide:

$$(HOOCCH_2CH_2CO)_2O_2 + H_2O \rightarrow HOOCCH_2CH_2COOH + HOOCCH_2CH_2COOOH$$

Stabilized succinyl peroxide compositions have been marketed for the disinfection of medical instruments.

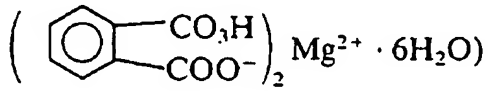
6.4.3 *Aromatic compounds*

Aromatic peroxyacids may be considered as derivatives of perbenzoic acids:



This compound is unstable at room temperature, both as a solid and in solution. However, many derivatives have been evaluated for characteristics such as stability and solubility as well as antimicrobial activity. Some particularly suitable compounds have been identified as 4-*t*-butyl-, 3-chloro-, 4-methyl- and 4-methoxyperbenzoic acids [57]. Sporicidal mixtures consisting of such compounds

in an aqueous alcoholic medium have also been described [58]. However, most development of this class of compounds has occurred with monoperoxyphthalic acid in the form of its magnesium salt. This compound is magnesium monoperoxyphthalate hexahydrate:



This is available commercially under the tradename Interox H48 and is both an aromatic peroxycarboxylic acid and the magnesium salt of an aromatic carboxylic acid. The result is a compound which is a stable, safe, water-soluble solid. This compound displays broad-spectrum antimicrobial activity, being effective against bacteria, yeasts and bacterial spores [59]. Although sporicidal activity is slow at 22 °C, a modest increase in temperature or use in alcoholic solution results in much greater potency. As with peracids in general, activity is reduced by only a small extent in the presence of organic matter. Slightly acidic solutions, such as are formed naturally upon the dissolution of the compound in water, favour antimicrobial activity. Various applications of this compound have been developed, particularly as part of formulated solid products for consumer use including nappy sanitizers and cleaners for hard surfaces, such as those found in bathrooms and kitchens. Other applications include use with anionic surfactants in a powder which, when dissolved in water, can be used to disinfectant floors, walls and similar surfaces in hospitals. This mixture is effective not only against bacteria and fungi but also viruses including the hepatitis B and HIV (AIDS) viruses.

6.4.4 Activated peroxide systems

In addition to the peracids themselves, much work has been carried out with activator systems, i.e. chemicals that react with hydrogen peroxide to form a more active peracid *in situ*. Many of these form peracetic acid *in situ* and thus offer the opportunity to utilize the biocidal properties of peracetic acid without the difficulties involved in the handling and transport of paracetic acid solutions. However, although many activators have been patented, few have found commercial applications as disinfectants. One reason for this may be that most systems have been designed for use under the alkaline conditions employed in the washing of clothes, the aim being to enhance bleaching at lower washing temperatures. Antimicrobial activity and peracid stability are favoured by mildly acidic conditions and thus bleach activators are generally unsuitable for use as disinfectants. Some activators suggested for use as biocides include α-acetoxy-α-methyl-*N*,*N*-diacetylmalonamide [60], 4-benzoyloxybenzenesulphonic acid [61] and *N*,*N*,*N*,*N*-tetraacetylethylene diamine [62].

6.5 Persulphates

The alkali metal perdisulphates, which contain the $S_2O_8^{2-}$ ion, are strong oxidants but display little antimicrobial activity, probably as a result of high kinetic energy barriers. No commercial biocides containing perdisulphates are currently available although a mixture containing ammonium perdisulphate has been described for the cleaning and disinfection of diary equipment [63] whilst the use of sodium perdisulphate in the treatment of swimming pool water has been reported [64].

Of far greater interest is Caro's acid triple salt, represented by the formula $2KHSO_5.K_2SO_4.KHSO_4$. Caro's acid or monoperoxosulphuric acid, H_2SO_5, can be made from hydrogen peroxide and sulphuric acid. Partial neutralization with potassium hydroxide under controlled conditions results in the formation of the triple salt, which is a stable, water-soluble solid. Caro's acid triple salt is active against both viruses [65] and bacteria but, by itself, displays no activity against yeasts or other fungi [66]. However, mixtures can be prepared that are both fungicidal and sporicidal, although the nature of the observed synergism with, for example, alcohols is unknown. Activity tends to be greater against gram-negative bacteria than against gram-positive organisms and acidic pH conditions enhance disinfection. The salt itself is naturally acidic, a solution containing 20 g/l in demineralized water having a pH of 2.2. Other noticeable features of the salt are that the final decomposition products are innocuous inorganic compounds and that, in contrast to the organic peroxyacids, stable aqueous solutions containing no hydrogen peroxide can be prepared since no hydrolysis leading to reversion to hydrogen peroxide takes place. Solution stability is generally greatest at pH values below 5.

A number of applications for Caro's acid triple salt (which is available commercially under a range of tradenames such as Curox) have been developed, including a water treatment process in which a quaternary ammonium compound and copper and/or silver salts are also used [67]. Most patents describe systems in which the oxidizing power of the peroxygen is not used directly for antimicrobial purposes but those in which chloride or bromide ions are oxidized to the corresponding hypohalous acid:

$$H^+ + HSO_5^- + Br^- \rightarrow HSO_4^- + HOBr$$

Such systems have found applications in a range of areas, often as a more acceptable alternative to organohalogen compounds, including swimming pool treatment [68], the control of slimes in aqueous systems [69], the sterilization of soft contact lenses [70] and for the sanitization of babies' nappies [71].

6.6 Other compounds

Several other types of peroxygen compound have specialized uses as antimicrobial agents. Calcium peroxide, CaO_2, has been reported to alleviate

microbial inhibition of cereal seed establishment [72]. Benzoyl peroxide, $C_6H_5COOOCOC_6H_5$, is in widespread use in dermatological formulations for the treatment of acne. Synergistic mixtures of hydroperoxides such as *t*-butyl hydroperoxide, $(CH_3)_3COOH$, and organic biocides such as phenols have been suggested as a means of preventing microbial attack on aviation and marine fuel [73], cutting oils and timber [74].

6.7 Conclusions

In a diverse range of industries, the use of hydrogen peroxide, peracetic acids and other peroxygen compounds will reflect an even wider spectrum of application, exploiting their antimicrobial properties as algicides, bactericides, fungicides, slimicides, sporicides, ovicides and viricides. The increasing enforcement of legislation to decrease pollution, increase sanitary conditions and improve quality control standards of many food and beverage products, coupled with the need for biocides and sterilants that are environmentally acceptable, will favour the advantages offered by peroxygen compounds. While emphasis will continue in the reduction of levels of harmful microorganisms in many areas of industry, attention will also be focused on the replacement of common chlorine and sodium hypochlorite based sanitizers and formaldehyde – the former because they may produce dangerous chlorinated byproducts and the latter because it may prove hazardous to health and safety.

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